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Review Article

Gene modulation and immunoregulatory roles of Interferonγ

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ABSTRACT

Interferon-gamma (IFNγ) is a central regulator of the immune response and signals via the Janus Activated Kinase (JAK)-Signal Transducer and Activator of Transcription (STAT) pathway. Phosphorylated STAT1 homodimers translocate to the nucleus, bind to *Gamma Activating Sequence* (*GAS*) and recruit additional factors to modulate gene expression. A bioinformatics analysis revealed that greater number of putative promoters of immune related genes and also those not directly involved in immunity contain *GAS* compared to response elements (RE) for Interferon Regulatory Factor (IRF)1, Nuclear factor kappa B (NFκB) and Activator Protein (AP)1. *GAS* is present in putative promoters of well known IFNγ-induced genes, *IRF1*, *GBP1*, *CXCL10*, and other genes identified were *TLR3, VCAM1, CASP4*, etc. Analysis of three microarray studies revealed that the expression of a subset of only *GAS* containing immune genes were modulated by IFNγ. As a significant correlation exists between *GAS* containing immune genes and IFNγ-regulated gene expression, this strategy may identify novel IFNγ-responsive immune genes. This analysis is integrated with the literature on the roles of IFNγ in mediating a plethora of functions: anti-microbial responses, antigen processing, inflammation, growth suppression, cell death, tumor immunity and autoimmunity. Overall, this review summarizes our present knowledge on IFNγ mediated signaling and functions.

1. The Interferon family

Interferons (IFN) were first discovered by Isaacs and Lindemann in 1957. The initial experiments were performed in chick chorioallantoic membranes in a nutrient fluid where the addition of influenza virus stimulated the production of a protein which interfered or prevented viral replication. In 1965, IFNγ was discovered as a viral inhibitory protein produced by lymphocytes in response to mitogen stimulation. Though initially named immune IFN, it was later renamed IFNγ. Subsequently, different types of IFN have been identified and categorized into three families: Types I–III [1]. The focus of this review will be on IFNγ, a Type II IFN.

IFNγ is widely distributed, from puffer fish to humans and plays pivotal roles in host defense (reviewed in [2–4]). The importance of IFNγ is reflected in patients lacking IFNγ or its receptors or its key signaling molecules. These patients display increased susceptibility to microbial infections, e.g. fatal dissemination of *Bacillus Calmette-Guerin* during infancy [5,6]. IFNγ has been used to treat several diseases and malignancies: chronic granulomatous disease (CGD) is an inherited disorder of leukocyte function caused by defects in nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, the enzyme complex responsible for phagocyte superoxide generation. Recurrent life-threatening bacterial and fungal infections, as well as abnormally exuberant inflammatory responses, are common in CGD. Infections are dramatically reduced by prophylaxis with antibiotics, antifungals, and IFNγ. In fact, the prolonged use of IFNγ in patients with CGD appears to be safe and shows persistent reduction in the frequency of serious infection and mortality [7]. Similarly, in severely immunosuppressed patients suffering from acute myelogenous leukemia, IFNγ is being used for treatment of invasive fungal infections. Also, in difficult-to-treat fungal infections, the addition of IFNγ appears to improve the outcome of the treatment regime [8]. These studies underscore the importance of IFNγ in host defense and its potential as a therapeutic agent for selected maladies.

Abbreviations: AP1, Activator Protein 1; APC, antigen presenting cells; CGD, chronic granulomatous disease; EAE,experimental autoimmune encephalomyelitis; ER, endoplasmic reticulum; GAS, Gamma Activation Sequence; GBP, guanylate-binding proteins; IDO, Indoleamine 2,3 dioxygenase; IFN, Interferon; IFNγ, Interferon-gamma; IFNGR, IFNγ receptor; iNOS, immune nitric oxide synthase; IRF, Interferon Regulatory Factor; IRG, immunity regulated GTPase; ISG, IFN-stimulated gene; ISRE, interferon stimulated response element; JAK, Janus Activated Kinase; JNK, c-Jun N-terminal kinases; MAPK, mitogen activated protein kinase; MHC, Major Histocompatibility Complex; MHC-I, MHC encoded class I molecules; MHC-II, MHC encoded class II molecules; NADPH, nicotinamide adenine dinucleotide phosphate; NFκB, Nuclear factor kappa B; NOD, non-obese diabetic; NK, natural killer; PI3K, phosphatidylinsositol-3-kinase; PKC, Protein kinase C; RE, response element; RNI, reactive nitrogen intermediates ROS, Reactive Oxygen Species; SH2, Src homology 2; SHP2, SH2 domain-containing tyrosine phosphatase; SOCS, suppressor of cytokine signaling; STAT, Signal Transducer and Activator of Transcription; TAP, transporter associated with antigen processing; TAPASIN, TAP-associated glycoprotein; T-BET, T-box expressed in T cells; Th, T helper; TLR, Toll-like receptors; Treg, T regulatory; VLIG, very large inducible GTPase.

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2. IFNγ signaling

IFNγ is secreted by activated T cells, natural killer (NK) cells and macrophages. Mature IFNγ is a protein of 143 amino acids with a molecular weight of ~20 kDa. IFNγ is an acid-labile and dimeric cytokine and each monomer consists of a core of six α-helices and an extended unfolded sequence in the C-terminal region [9]. The biologically active dimer is formed by anti-parallel inter-locking of two monomers [10]. The binding of IFNγ to its receptor activates the JAK-STAT pathway which modulates the transcriptional activation of several genes and mediates diverse biological responses (Fig. 1). The IFNγ receptor consists of two subunits, IFNγ receptor (IFNGR)1 and IFNGR2, and each molecule interacts with a member of the JAK family, which are non-receptor protein tyrosine kinases. JAKs phosphorylate receptors and transcriptional coactivators known as STATs (reviewed in [11]).

IFNγ binds to its receptors with high affinity and in a species-specific manner, i.e. human IFNγ does not bind to the mouse IFNGR and vice versa. IFNGR1, the larger subunit, is required for ligand binding and its carboxy terminus is involved in binding to JAK1 and phosphorylated STAT1. The smaller subunit, IFNGR2, is required for signaling and contains the JAK2 binding site. After engagement of the IFNGR with IFNγ, phosphorylation of the receptor generates a binding site for STAT1 via its Src homology 2 (SH2) domain. Phosphorylation of STAT1 on Tyr701 results in the formation of STAT1 homodimers, known as Gamma-Activated Factor, which translocate to the nucleus, bind to *GAS* and enhances transcriptional activation by recruiting several transcriptional coactivators. (Fig. 1). For optimal activity, STAT1 needs to be also phosphorylated on Ser727 [12]. The importance of this phosphorylation is demonstrated in mutant mice expressing the Ser727Ala mutant of Stat1, which shows increased mortality upon infection with *Listeria monocytogenes* [13]. Importantly, *Stat1*$^{-/-}$ mice do not signal via IFNαβ or IFNγ and are extremely susceptible to microbial infections [14].

IFNγ signaling has been studied for several years; however, accumulating evidence clearly demonstrates the roles of other pathways. Activation of phosphatidylinsositol-3-kinase (PI3K) by IFNγ seems to have important functional consequences in IFNγ-inducible transcriptional regulation. Inhibition of PI3K blocks the IFNγ-dependent phosphorylation of STAT1 on Ser727 and reduces STAT1 driven transcription. IFNγ activation of the PI3K pathway also leads to activation of Protein kinase C (PKC) ε, which activates the mitogen activated protein kinase (MAPK) pathway, and transcriptional activation of *STAT1* [15]. IFN are also involved in transcriptional activation of PKCθ, which in turn activates MAPK kinase 4 [16]. In addition, PKCδ is activated by treatment of cells with IFNγ and has been shown to associate with STAT1 [17]. IFNγ activates various MAPK pathways leading to regulation of cell growth, differentiation, apoptosis, etc. In response to stress (e.g. UV or LPS), STAT1 is phosphorylated on Ser727 by p38 MAPK [18]. In bone marrow derived primary macrophages, IFNγ induces p38 MAPK and increases the expression of genes involved in chemotaxis and inflammation, e.g. *CXCL10* (*IP10*), *TNFα* and *NOS2* (*iNOS*). Although Extracellular signal-regulated kinases and c-Jun N-terminal kinases (JNK) are also activated by IFNγ, the extracellular signal-regulated kinases pathway has modest effects on pro-inflammatory gene expression whereas JNK regulates the expression of genes involved in antigen presentation, e.g. *CIITA*. Therefore, IFNγ activation of MAPK selectively modulates macrophage responses [19]. Further studies are required to fully comprehend IFNγ signaling and the cross talk that occurs across pathways.

The activation of STAT1 by IFNγ is not continuous and is inhibited after some time, suggesting the presence of negative

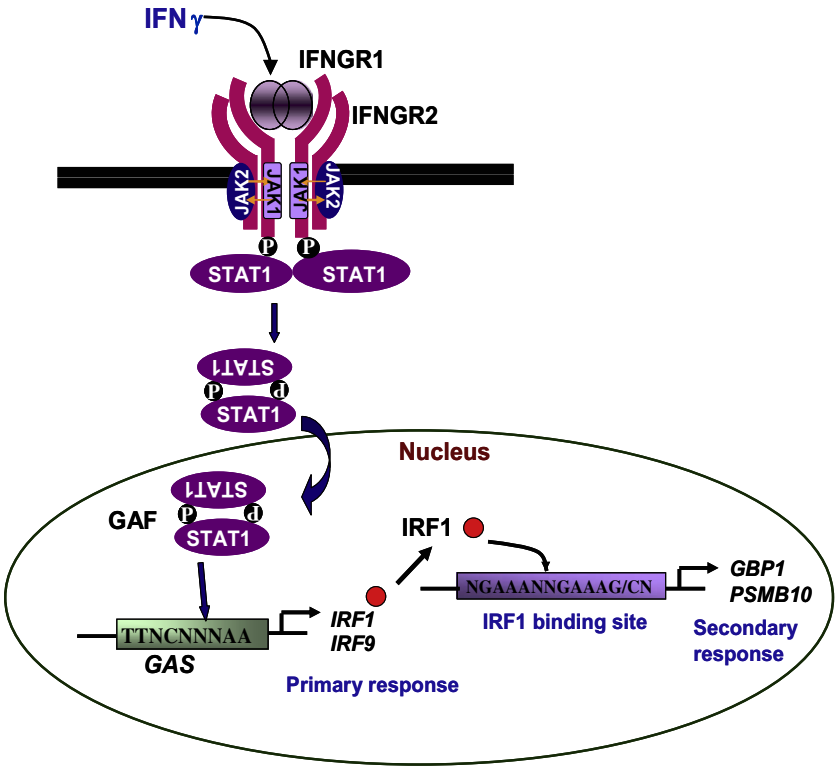

Fig. 1. The IFNγ signaling pathway. Binding of IFNγ results in receptor oligomerisation followed by trans-phosphorylation and activation of JAK1 and JAK2. Activated JAKs phosphorylate IFNGR1 resulting in docking of STAT1. Phosphorylated STAT1 homodimerizes to form Gamma-Activated Factor which binds to *GAS* located in the promoters of several primary response genes and increases transcription by recruiting several coactivators. Upon induction, the transcription factor IRF1 binds to *ISRE* and enhances transcription of several secondary response genes.